Biological Control: Benefits and Risks

Edited by

Heikki M. T. Hokkanen
University of Helsinki, Finland

and

James M. Lynch
University of Surrey, Guildford, UK

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Suppressiveness of soils to invading micro-organisms

Claude Alabouvette and C. Steinberg

Introduction

Many attempts have been made to control soil-borne plant pathogens and to improve the growth of plants by inoculation of seed or soil with selected strains of micro-organisms; mainly bacteria. More recently, selected bacteria that are expected to degrade xenobiotics have been suggested for the bioremediation of polluted sites (Short *et al.*, 1990). Nowadays, risk assessment studies also need to address the fate of engineered telluric soil and non-telluric soil bacteria (Tiedje *et al.*, 1989; Doyle *et al.*, 1991).

Besides increased crop yields claimed by Russian workers (Schroth and Becker, 1990), most of the tentative applications of micro-organisms in agricultural soils have failed. The widely practised inoculation of legume seeds with *Rhizobium* spp. is one of the few examples of success of application of micro-organisms to improve crop yield (Stacey and Upchurch, 1984). Application of the strain K84, and more recently of the modified strain K1026, of *Agrobacterium radiobacter* to control crown gall of plants is one of the few examples of a biological control method commercially applied with some success in several countries (Ryder and Jones, 1990). In most cases, the beneficial effects expected from the microbial inoculation are not consistently reproduced under field conditions. The poor survival of the introduced micro-organisms in soil is the main explanation for these failures. In fact, the population density of the introduced micro-organisms decreases to the limit of the carrying capacity of the soil. Moreover, the soil represents very heterogeneous environments in which the introduced micro-organisms must find suitable habitats, some of which are very strain specific (Hattori and Hattori, 1976). It is often admitted that exogeneous populations of micro-organisms are difficult to establish in the soil in such a way that they express the specific activity for which they have been selected. Therefore, many studies aim to improve the saprophytic or rhizospheric competence of beneficial micro-organisms in the soil environment (Ahmad and Baker, 1987).

Contrasting with this belief is the fact that it is almost impossible to eradicate diseases due to soil-borne plant pathogens, which indicates that these micro-organisms are able to survive in soil for long periods and under various climatic conditions.

Currently, there is a tendency to believe that genetically engineered micro-organisms will help to improve the efficacy of antagonists or plant-growth-promoting rhizobacteria (PGPR) and many scientists expect the use of such modified micro-organisms in agriculture. Even though insertion genes will probably cause micro-organisms to have reduced ecological competence (Schroth and Becker, 1990), there is great concern about the risk of losing control of these manipulated organisms and of the transmission of exogenous genes to wild micro-organisms in soil. Most of the risk assessment studies only consider transformed micro-organisms, but there is also a need to improve knowledge of the ecology of naturally occurring micro-organisms. Plant pathologists studying soil-borne plant pathogens are among the microbiologists with the longest

experience in the field of microbial ecology. They have been accustomed to infesting soils with pathogens to reproduce disease symptoms and with antagonists to control diseases. Twenty-five years of studying soil that is naturally suppressive to diseases induced by soil-borne pathogens has produced a large amount of data related to the interactions between the pathogens and the antagonistic micro-organisms, and between the microbiota and the abiotic characteristics of the soils (Cook, 1990). The existence of soils that do not enable disease expression even after infestation with the pathogen demonstrates that the soil environment can suppress either the establishment or the activity of an introduced population of micro-organisms (Alabouvette, 1990). If soil suppressiveness to diseases is mainly due to microbial interactions between the pathogen and the antagonistic microflora, then it is not independent from the abiotic characteristics of the soils. Indeed, soils suppressive to fusarium wilts provide an example where the suppression of the disease is not necessarily due to the suppression of the pathogen; however, pathogen survival and disease suppressiveness are under the indirect control of the abiotic soil properties. Therefore, it is interesting to review the subject of suppressiveness of soil to the invasion of micro-organisms in relation to the concepts linked to soil suppressiveness of fungal diseases.

Survival of microbial populations introduced into soils

Studying the survival kinetics of a microbial population introduced into soil requires accurate methods to assess the population density. Many techniques based on direct or indirect assessments have been developed; all of them have some inconveniences that makes it difficult to compare data obtained by different workers, and different techniques. However, it appears that most of the micro-organisms studied are able to survive for some time in soil. The following examples illustrate different kinetics of survival after experimental introduction of bacteria or fungi into soil; but this is not intended to be an exhaustive review of the literature.

Bacteria

Assessing the fate or predicting the activity of a bacterial population introduced into soil appears to be suited to a case by case study, and is difficult to generalize. However, some general principles can be mentioned as will be shown by inspection of the kinetics of different bacterial species introduced into soil.

A comparison of the population dynamics of various telluric or non-telluric strains of bacteria in a clay loam soil (clay, 32%; silt, 36%; sand, 32%; pH 6.5) in microcosms (Richaume *et al.*, 1990) resulted in a primary discrimination of two groups of bacteria : the surviving one and the non-surviving one (Fig. 1.1). When inoculated at a high density, close to 10^8 bacteria/g soil, the various telluric strains first rapidly declined, then established at a lower level of 10^5 bacteria/g soil (*Azospirillum lipoferum, Agrobacterium radiobacter* or 10^6 bacteria/g soil (*Pseudomonas aeruginosa*) depending on the bacterial species. The slight increase of the *Bradyrhizobium japonicum* and *Agrobacterium radiobacter* densities observed on the days following the inoculation was interpreted by the authors as being growth at the expense of internal reserves such as poly-β-hydroxybutyrate because they had been grown on a rich culture medium. On the other hand, the population of

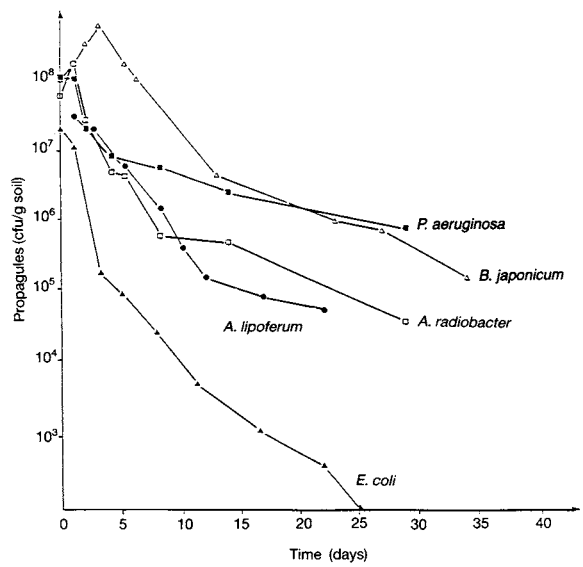

Fig. 1.1 Population dynamics of various bacterial populations introduced into a clay loam.

Escherichia coli declined rapidly and was no longer detectable after 25 days, as has often been reported (Devannas *et al.*, 1986; Recorbet *et al.*, 1992*a*).

The secondary discrimination obtained from these results concerned the telluric species. After one month of incubation, each of them persisted at a specific level. Moreover, Crozat *et al.* (1987) showed that strain G2 sp. of *Bradyrhizobium japonicum* established itself at a level 3 times higher (significant: $P > 0.05$) than the level reached by G49, a strain of the same species, inoculated in the same clay loam soil. The results indicated a soil–bacterium interaction which was strain dependent.

Nevertheless, the general feature of these kinetics was the rapid decline of the bacterial densities, which did not occur in sterile soil (Steinberg *et al.*, 1987) or with fungi (Couteaudier and Alabouvette, 1990). The question that arose was: why did the bacteria not maintain a high density following the inoculation? Two explanations can be provided depending on, once again, the type of bacteria.

1. The ability of the strain to compete successfully when introduced into the soil plays a major role in the establishment of non-telluric bacteria (Recorbet *et al.*, 1992*b*), as well as of engineered bacteria, compared with the parental strain (Van Elsas *et al.*, 1991).
2. With regard to telluric non-manipulated bacteria, it has been widely demonstrated that protozoan predation is the most important factor in the regulation of introduced populations (Habte and Alexander, 1977; Steinberg *et al.*, 1987). This regulating factor could explain why the equilibrium level reached by the bacteria was independent of the inoculum densities at which they were introduced. However, because this equilibrium level differed between strains, it must be stated that very specific interactions between the bacteria and the environment occurred.

Both biotic and abiotic factors acted rapidly because the decline generally occurred in less than one month. Nevertheless, field experiments carried out with *B. japonicum* strains showed that the population density remained stable at around 10^3 or 10^4 bacteria/g soil after 10 years in the presence or absence of soybeans. It is noteworthy that not only was the bacterial density stable during these years but some genetic and physiological characteristics were also. Isolates of one of these strains were recovered by plant or immunological trapping and no genetic or physiological modifications were detected as compared with the parental strains retained in the laboratory (Brunel *et al.*, 1988).

Fungi

Most of the studies are related to the survival of soil-borne plant pathogens and their antagonists. Only two examples are given to show that fungi are able to survive for long periods in soil not only as resting structures but also as active saprophytes.

Studying the population dynamics of a strain of *Trichoderma harzianum* introduced in a field soil, Davet (1983) demonstrated that the fungus was able to survive saprophytically for 3 years (Fig. 1.2). Grown on straw, the inoculum was applied at a rate of 2 kg/m^2; the population of *Trichoderma* reached a density greater than 1×10^5 colony forming units (cfu)/g soil immediately after inoculation, then decreased slowly. At the end of the experiment, the population density of *Trichoderma harzianum* was still 10 times greater in the treated plot than in the control, where the wild population of *Trichoderma* spp. established at 3×10^3 cfu/g soil. Davet (1983) was also able to demonstrate that the decline of the population during summer was due to drought; the population increased again in the autumn after a period of rain.

In contrast to these experimental conditions in

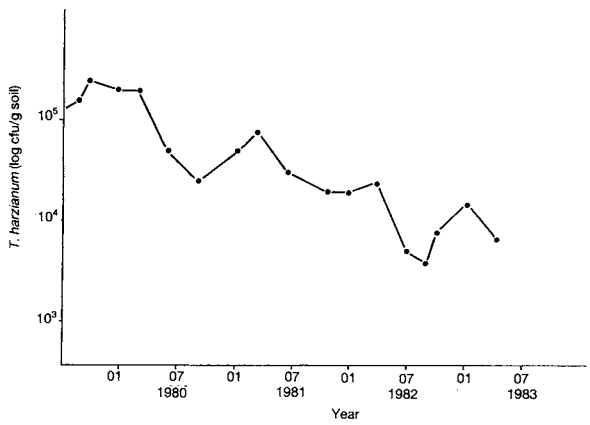

Fig. 1.2 Population dynamics of *Trichoderma harzianum* introduced into a sandy loam.

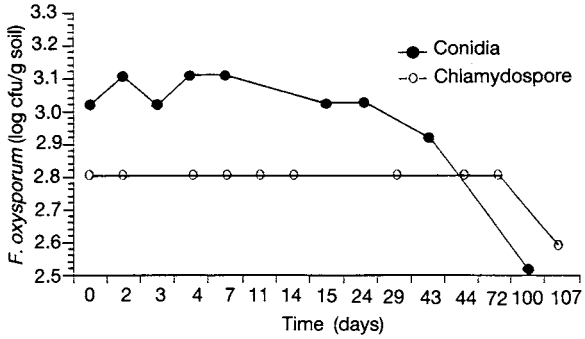

Fig. 1.3 Population dynamics of *Fusarium oxysporum* f.sp. *lini* introduced either as chlamydospores or microconidia into a clay loam.

an open field, Couteaudier and Alabouvette (1990) studied the population dynamics of a strain of *Fusarium oxysporum* f.sp. *lini* introduced into raw soil under well standardized conditions. Not only the chlamydospores but also the microconidia were able to survive for at least 100 days. Introduced at the initial concentration of 1.4×10^3 cfu/g soil, the density of the chlamydospore population remained stable for 72 days but fell significantly between 72 and 107 days (Fig. 1.3). The population dynamics of the conidial inoculum introduced at 3×10^3 cfu/g soil were similar to those described with chlamydospores. However, the decline of the population was greater and after 100 days of incubation only 21% of the conidia had survived compared with 70% of the chlamydospore inoculum. When *F. oxysporum* f.sp. *lini* was introduced at higher inoculum concentrations, i.e. 1.5×10^6 and 3×10^6 cfu/g soil respectively for chlamydospores and conidia, the survival kinetics were similar to those described for the lower inoculum concentration. Therefore, after 100 days of incubation in soil, the higher and the lower inoculum concentrations were significantly different; this indicates that the mechanisms of regulation of the microbial populations described for other micro-organisms, especially predation, did not apply to *Fusarium oxysporum* (Levrat *et al.*, 1991).

Capacity of soils to support the survival of introduced micro-organisms

The previous examples indicated that most bacteria and fungi are able to survive for long periods in soils. However, soils may differ greatly with respect to their abiotic (texture, pH, etc.) and biotic characteristics. Therefore, the survival kinetics of a given strain of micro-organism may be different depending on the soil type.

Survival of one bacterial strain in various soils

As previously mentioned, the establishment of a bacterial population depended on a soil–bacteria interaction that seemed to be strain dependent. In fact, this highly specific interaction could also be qualified as a soil dependent interaction.

The survival of the *Pseudomonas fluorescens* WCS374ln5 strain in a silt loam was significantly ($P=0.05$) better than in a loamy sand. On the other hand, an introduced population of the *Bacillus subtilis* F6 strain established in very few days at a similar level both in the loamy sand and in the silt loam (Van Elsas *et al.*, 1986). The *Bacillus* population consisted mainly (60–100%) of spores when established at the stabilized level of 5×10^3 cfu/g soil. Because of their different cell wall properties, $Gram^+$ bacteria and $Gram^-$ bacteria must react differently with respect to biotic and abiotic soil components. Moreover, the ability of $Gram^+$ bacteria to form spores renders them less sensitive to regulating factors. These two types of bacteria should be considered separately.

Moffett *et al.* (1983) also found that the survival of two strains of *Pseudomonas solanacearum* were greater in a clay loam than in a sandy loam. All of these findings suggest that bacteria are better able to survive in clay soils than in sandy soils. Indeed, Heijnen and Van Veen (1991) demonstrated a protective effect of bentonite clay and to a lesser extent kaolinite, against predation of *Rhizobium*. This effect was attributed to modifications in the pore-size distribution. Previously, Stotzky and Rem (1966) suggested the possible relationship between clay minerals and activity and population dynamics of bacteria in soils. Even though Stotzky and Rem, were referring to naturally occurring micro-organisms, their hypothesis could be extended to introduced micro-organisms.

Corman *et al.* (1987) modelled the survival kinetics of three strains of *B. japonicum*. Each of them was inoculated into three soils of different textures (a sandy soil, a silty sand and a silt loam). The highest rate of decrease was always observed

Table 1.1. Estimation of rates of decline (days^{-1}) obtained for each kinetic by the sum of the least squares technique (*B. japonicum*)

	Soils		
Strain	Sandy soil	Silty sand	Silt loam
G2sp	1.2	0.16	1.1
G49	0.64	0.1	0.37
GMB1Ka	0.41	0.34	0.19

in the sandy soil while only for strain GMB1Ka the lowest rate was obtained in the silt loam. Strain G49 and strain G2sp declined significantly more rapidly in the silt loam than in the silty sand (Table 1.1). These results suggested that clays were not the only key factor involved in the survival and activity of bacteria.

The aim of inoculation of soil or seed with bacteria is to obtain some beneficial effect (increased yield, biological control, bioremediation), therefore, not only the ability to survive but mainly the ability of the introduced population to express its activity in soil must be assessed. In other words, the level at which the population density stabilizes should result in the expected beneficial effect. This is the case for seed inoculation of legumes with *Rhizobium* or *Bradyrhizobium*; N_2 symbiotic fixation is efficient after the introduced bacterial population has stabilized in soil.

Van Elsas and Heijnen (1990) gave examples of applications of selected or engineered inoculated bacteria, but without any reference to the real agronomic benefit. Nevertheless, because of the potential applications of inoculation of soil with bacteria, the influence of such an inoculation on the indigenous populations has to be assessed. Most authors conclude their papers with the assertion that this should be checked but this is done very rarely. Doyle *et al.* (1991) demonstrated that a genetically manipulated strain of *Pseudomonas putida* (strain pp.0301 (pR0103)), in the presence of the substrate on which its novel genes can function, was capable of inducing measurable variations in the composition of the indigenous fungal and bacterial populations.

The expression of the biological activity at a significant level from an agronomical point of view depends on both the survival density and the physiological state of the introduced micro-organisms after they have established. The results presented here show that this establishment is influenced by a very specific soil–bacteria interaction. This soil–bacteria interaction, in turn, depends on complex processes that involve bacterial cell surface properties, soil, biological and physical components and the interaction between abiotic and biotic factors.

Fungi

Davet (1983) compared the survival kinetics of a strain of *Trichoderma aureoviride* in two field soils. The soil from Dijon was a clay loam of pH 6.3; the soil from Perpignan was a sandy loam of pH 5.9. The fungus was grown on straw and introduced into soil at the rate of 450 g/m^2. After soil infestation, the first estimation of the population density gave similar results for both soils. However, the population rapidly evolved differently in the two soils and the population density was higher in soil from Dijon than in soil from Perpignan (Fig. 1.4). This difference was evident until the end of the experiment and demonstrated that the carrying capacity of the soil from Dijon was greater than that of the soil from Perpignan; this could be attributed to the greater clay content of the soil from Dijon.

Amir and Alabouvette (1993) compared the population dynamics of a strain of *F. oxysporum* f.sp. *lini* introduced at the same inoculum density

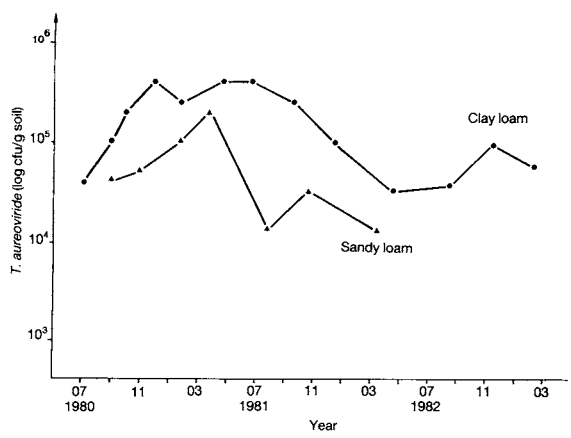

Fig. 1.4 Population dynamics of *Trichoderma aureoviride* introduced into a sandy loam and a clay loam.

into a sandy soil (sand 96%, silt 1.5%, clay 2.5%) and a clay loam soil (sand 19%, silt 44%, clay 37%). They observed that the population density dropped in both soils, but the decline of the population was greater in the sandy than in the clay soil. After 60 days of incubation, the residual populations were significantly different and represented 12% and 44% of the initial populations in the sandy and the clay soil respectively (Fig. 1.5). However, these two soils were not only different in texture; the sandy soil was conducive to fusarium wilt of flax compared with the clay soil, which was suppressive (Fig. 1.6). Although the clay soil supported larger populations of the pathogen, it had a lower inoculum potential than the sandy soil and therefore showed a lower disease incidence.

These results are not in accordance with previous data published by Alabouvette *et al.* (1985) indicating that the population dynamics of *F. oxysporum* f.sp. *melonis* were similar in a conducive and in a suppressive soil from another origin. The density of the introduced species remained almost stable for more than 1 year and was not significantly different between the soils. However, it must be noted that these two soils contained 18.4 and 15.5% clay respectively; their texture was closer to that of the clay soil than to that of the sandy soil described in the previous example.

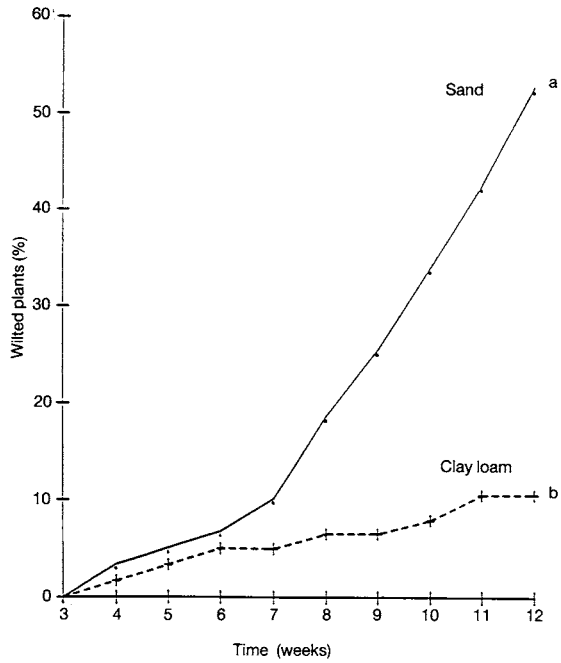

Fig. 1.6 Percentage of wilted plants after infestation of a sandy soil and a clay loam with *F.o.* f.sp. *lini* (4×10^4 cfu/g soil).

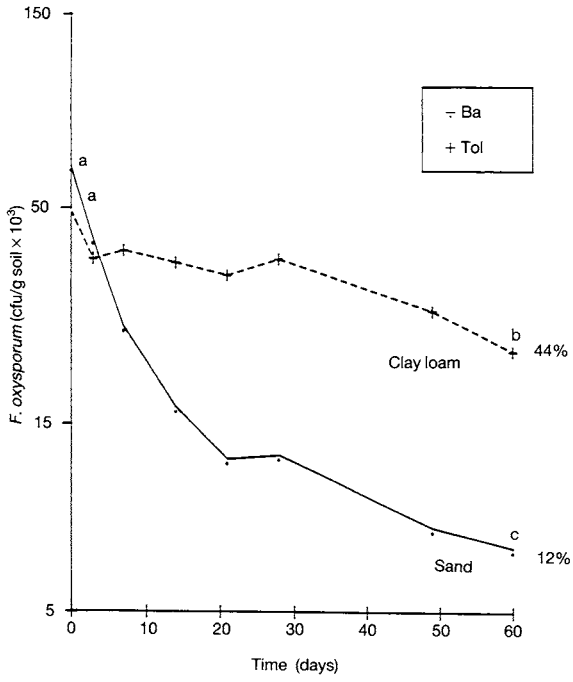

Fig. 1.5 Population dynamics of *Fusarium oxysporum* f.sp. *lini* introduced into a clay loam and a sandy loam.

Collectively, these results demonstrated that suppressiveness of soils to fusarium wilts is not correlated with the suppression of the pathogen in the suppressive soils. In suppressive soils, the introduced pathogen is able to survive at a high density without being able to induce the disease. On the contrary, in conducive soils, the density of the introduced pathogen may decrease significantly, but the remaining propagules are still able to induce the disease. This interpretation of our data leads to the hypothesis that the soil may exert an influence on the survival and on the activity of an introduced population of micro-organisms which is contrary to that expected. This ability of soils to control both the survival and the activity of a microbial population is not independent of their abiotic characteristics. Indeed, it is well established that clay soils support a larger biomass than sandy soils (Chaussod *et al.*, 1986) and clays have also been involved in soil suppressiveness of fusarium wilts (Stotzky and Martin, 1963).

Role of abiotic properties in mechanisms of soil suppressiveness

Amir and Alabouvette (1993) modified the texture of a sandy soil by the addition of fine particles of montmorillonite or talc (25% w/w). They observed that the survival kinetics of an introduced population of *F. oxysporum* f.sp. *lini* was modified; after 60 days of incubation, the inoculum density was not reduced to the same extent in the amended soil as in the control. The residual inoculum density represented 43% and 41% of the initial density compared with 2% in the control (Fig. 1.7).

The kinetics of CO_2 release after glucose amendment (1 mg/g soil) were significantly different (Fig. 1.8): after the addition of talc the amount of CO_2 released was lower than in the control, but it was enhanced after the addition of montmorillonite. These results suggest that montmorillonite enhanced the microbial activity of the soil, as already described by Stotzky and Rem (1966), and therefore increased the intensity of competition for nutrients between micro-organisms, leading to an increased level of fungistasis (Lockwood, 1988). This increased level of fungistasis could explain the better survival of the introduced population of *F. oxysporum* f.sp. *lini* in soil amended with montmorillonite. The beneficial effect of montmorillonite could be related to specific properties such as its large cation exchange capacity and its large surface area in comparison with talc.

Finally, the addition of talc or montmorillonite also modified the level of soil receptivity to fusarium wilt of flax. After soil infestation with 4×10^4 cfu/g soil of *F. oxysporum* f.sp. *lini*, disease incidence was significantly decreased after the addition of montmorillonite and increased after the addition of talc in comparison with the non-amended control. Talc made the soil more conducive to the disease and montmorillonite made it more suppressive (Fig. 1.9).

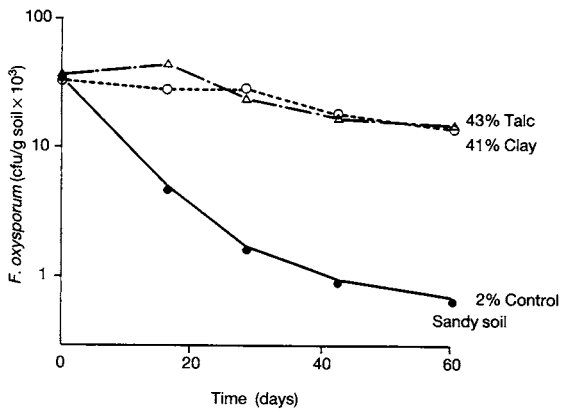

Fig. 1.7 Population dynamics of *F.o.* f.sp. *lini* introduced into a sandy soil following the addition of talc or montmorillonite (25% w/w).

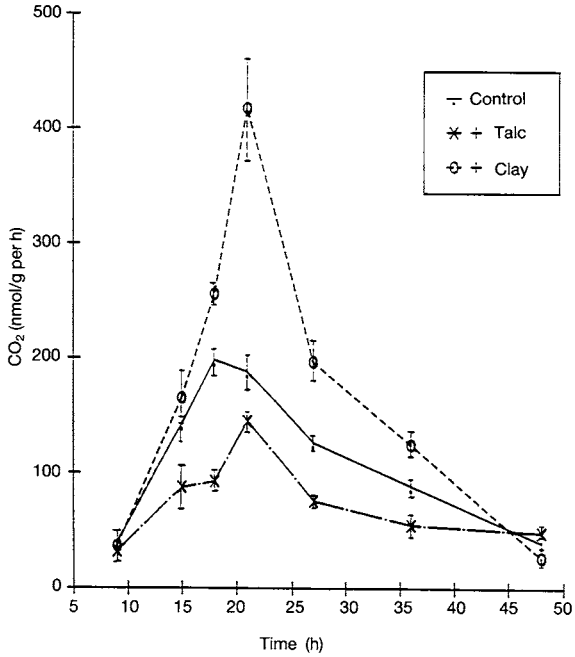

Fig. 1.8 Kinetics of CO_2 release from a sandy soil amended with glucose (1 mg/g) following the addition of talc or montmorillonite (25% w/w).

These results demonstrated that modification of the texture of the soil induced modification of the ability of the soil to support the survival of an introduced population of *Fusarium* and to control the infection activity of this inoculum.

Discussion

The experimental results reviewed in this paper demonstrate that most of the micro-organisms introduced into natural soil survive for weeks, or months, at levels high enough to be easily detected by the standard methods of enumeration.

The only exception concerns the non-telluric

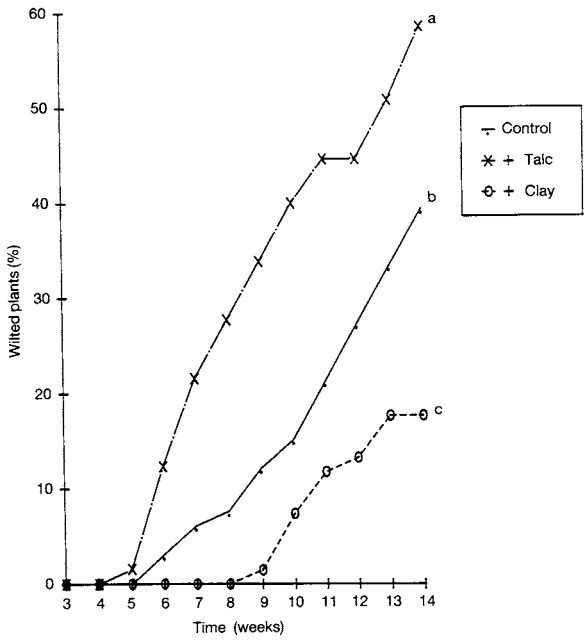

Fig. 1.9 Percentage of wilted plants after infestation of a sandy soil with *F.o.* f.sp. *lini* (4×10^4 cfu/g soil) following the addition of talc or montmorillonite (25% w/w).

bacteria such as *E. coli*, which are not adapted to the soil environment and cannot be detected after a few days or weeks. Generally, the decline of bacterial populations occurs faster than that of fungi. However, it is necessary to distinguish between bacterial species unable to produce spores and spore-forming bacteria, the latter being able to survive at higher densities. Their kinetics of survival can be compared with those of fungi, especially of fungi that form specialized survival structures such as chlamydospores and sclerotia. However, most of the soil-borne fungi are also able to survive as active saprophytes growing on organic matter. Some bacteria such as the symbiotic *Bradyrhizobium japonicum* are also able to survive for long periods in soils without forming resting spores.

The density of the introduced population of either bacteria or fungi usually reaches a plateau that corresponds to the carrying capacity of the soil. This carrying capacity is both strain and soil dependent. Little is known about the factors that determine this carrying capacity. One possible mechanism is that the bacterial populations are related to the nutrient status of the soil. The results from experiments reported in this paper show that the texture of the soil is also a factor that controls the capacity of soil to support the growth and activity of a microbial population. In general, the greater the clay and organic-matter contents of the soil, the better is the survival of the introduced population. However, most of the studies that deal with the behaviour of microbial populations in soil have been conducted after massive introduction of a given population. Only a few studies have considered the dynamics of a population introduced at a low inoculum density comparable to the levels that occur in natural soils. Therefore, it is not surprising to observe a quick decline of the population until it reaches the carrying capacity of the soil.

It must be stressed that most of the studies have only considered the kinetics of survival, i.e. the quantitative evolution of the population. However, studies of soils suppressive to diseases provide examples of soils that affect the activity of the population without necessarily affecting the density of the population. A soil can be conducive to the population of the pathogen and at the same time be suppressive to the disease induced by the pathogen. The soil affects the ability of the pathogen to survive and its ability to infect the host plant in various ways. This difference between suppressiveness towards a microbial population and its activity raises the question of what is the most important phenomenon to be considered. Obviously for the farmer, as well as for the plant pathologist, the suppression of the disease is the most important issue. If an antagonistic micro-organism or a PGPR is introduced into the soil, one expects its activity to result in a beneficial effect on the crop. In this case, the soil should support both the survival and the activity of the introduced population. And what about the risk of transferring exogenous genes to wild micro-organisms by the introduction of a transformed micro-organism into soil for an agronomical purpose? When the soil induces a rapid decline of the population one may assume that the risk is also declining. However, if the soil supports a high population density, and at the same time inhibits the activity of this population, the risk may be not related to the population density.

It is our feeling that most of the micro-organisms that could be introduced into soil will be able to survive for long periods, at least at low